

RICKETTSIA-ASSOCIATED MORTALITY OF THE YELLOW CLAM
MESODESMA MACTROIDES (BIVALVIA: MESODESMATIDAE)
IN SOUTHERN BRAZIL

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INTRODUCTION

The yellow clam *Mesodesma mactroides* (Deshayes, 1854) is an intertidal sandy beach bivalve that is distributed from the southeast of Brazil (Ilha Grande, Rio de Janeiro State, 23°S) to Argentina (Isla del Jabalí, Buenos Aires Province, 41°S) (Rios, 2009). Historically, the yellow clam had been considered an important economic resource (Coscarón, 1959). However, yellow clam populations collapsed as a result of overfishing that was associated with repeated mass mortalities in Brazil, Uruguay and Argentina in the mid-1990s (Odebrecht et al., 1995; Fiori & Cazzaniga, 1999; Cremonte & Figueiras, 2004). Presently, *M. mactroides* is identified as a threatened species with a critically endangered status (Fiori & Cazzaniga, 1999).

As more knowledge about the factors that can influence the population sizes of sea animals have become available, it has become clear that disease can drastically affect abundance (Sindermann, 1990). Major disease-causing agents of marine bivalves include viruses, prokaryotes, fungi, protists, and invertebrates, such as Digenea, Annelida and Copepoda (Kinne, 1983). According to Lauckner (1983), prokaryotes belonging to Rickettsiales are capable of causing disease and possibly death in marine bivalves from natural beds around the world and, both juveniles and larvae of bivalves are more susceptible to diseases caused by prokaryotes infections (Elston, 1999). Rickettsiae are small, pleomorphic coccobacilli considered to occupy a special taxonomic niche between bacteria and viruses (Lauckner, 1983). The first rickettsia-like organisms detected in molluscs were found in *Mya arenaria* from Chesapeake Bay (Harshbarger et al., 1977). According to Harshbarger et al. (1977), the

ribosome-rich, undulating rods, measuring 300 x 2,000 nm occurred in roundish inclusions, up to 100 µm in diameter and usually located singly in the epithelial-cell cytoplasm and lumen of digestive diverticula.

Although the yellow clam has important economic value, few histopathological studies of this species have been completed. For example, Cremonte & Figueras (2004) reported *Trichodina* sp., gregarins, coccidians and Turbellaria in yellow clams from the Argentinean coast, and Brusa et al. (2006) discovered a new species of Turbellaria, *Paravortex mesodesma*, in the intestinal lumen of *M. mactroides* from the Uruguayan coast. However, the parasites identified in these studies could not be responsible for yellow clam mortality, and there is little known about the pathological conditions of *M. mactroides* populations in Brazil.

Despite the increasing concern about diseases in edible bivalves on the Brazilian coast, particularly in Santa Catarina State in southern Brazil (da Silva et al., 2002, 2011; Sabry et al., 2011) and the northeastern states of Brazil (Azevedo et al., 2005; Sabry et al., 2007; Boehs et al., 2010), the occurrence and impact of bivalve diseases remain poorly studied. Furthermore, mortalities can occur without notice because a systematic surveillance programme is not currently in place (da Silva et al., 2011). Prior to this study, no histopathological research on the diseases of bivalves had been completed in the State of Rio Grande do Sul.

The programme to monitor the health status of *M. mactroides* began in 2011. The study area included Cassino Beach, which lies between Rio Grande City and Barra do Chuí on the south shore of Rio Grande do Sul State in southern Brazil. In October 2011 (austral spring), the local recruitment period of *M. mactroides*

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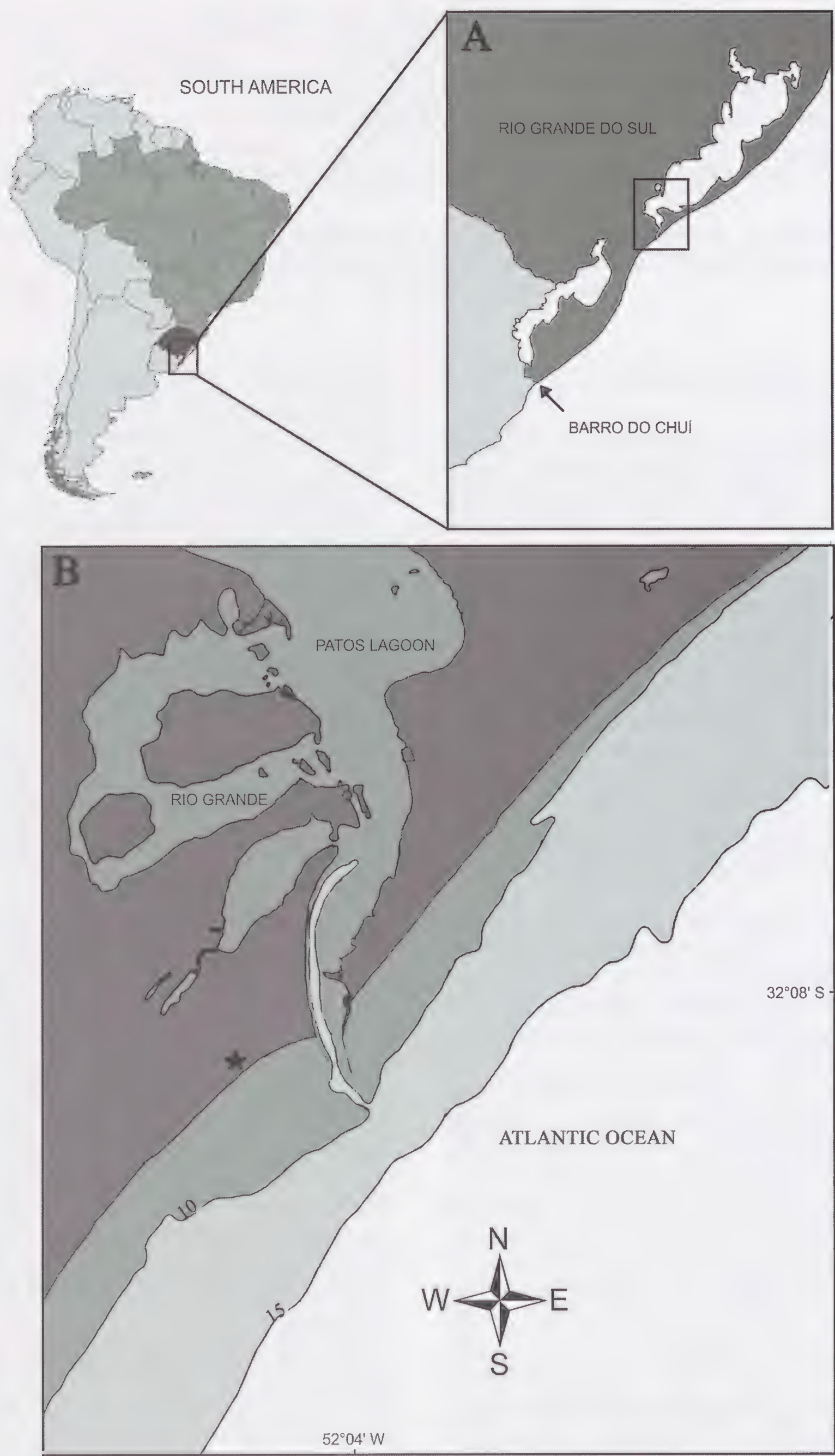


FIG. 1. Map of South America depicting the state of Rio Grande do Sul in southern Brazil. The star indicates the sampling location of moribund *Mesodesma mactroides* juveniles in Cassino Beach.

(Bergonci & Thomé, 2008), a large number of empty juvenile shells and moribund juveniles unable to burrow in the sand were observed at Cassino Beach. Initially it was thought that temperature or salinity could be responsible for the mortalities. However, abnormal fluctuations of these parameters were not observed (the temperature and salinity at the sampling time were 20°C and 30‰, respectively).

The objective of the present study was to determine the cause of the mortality event that affected juveniles of the yellow clam *M. mactroides* using histological assays for the detection of potential pathogens.

MATERIALS AND METHODS

Thirty moribund yellow clam juveniles (shell height ranging from 25 to 35 mm, mean = 29.35 ± 2.94 mm) were manually collected in the intertidal zone of Cassino Beach in southern Brazil (32°12'S, 52°11'W) (Fig. 1) in October 2011. The samples were transported to the laboratory as quickly as possible, and the soft parts of the moribund clams were carefully removed from their shells. They were fixed in Davidson's solution (Shaw & Battle, 1957) for one day and then stored in 70% ethanol.

Twenty whole individuals were processed with automated equipment (Lupe Tec, model PT 05, Brazil) and embedded in Paraplast®. Sections (3µm) were cut on a microtome (Lupe Tec, model MRP03, Brazil) and stained with haematoxylin and eosin (HE).

For transmission electron microscopy (TEM), small fragments of the intestines of ten clams were post-fixed in 2.5% glutaraldehyde and 1% OsO₄ in cacodylate buffer (0.05 M) (pH 7.4). The epithelial cells of the intestine were then dehydrated in ethanol and embedded in Epon 812 resin. Ultrathin sections were stained with uranyl acetate and lead citrate. Finally, the samples were examined with a microscope (JEOL, model 100S, Japan).

RESULTS

In 100% of the clams analysed, light microscopy revealed the accumulation of basophilic material infecting the epithelial cells of the intestine and the gills that corresponded to *Rickettsia* colonies (Fig. 2). No other parasites were recorded in the studied specimens.

Transmission electron microscopy of the infected midgut revealed varying numbers of rickettsiae in the lumen. Some were free in the lumen while others were intimately associated with the plasma membrane of the intestinal epithelial cells. In thin sections, the microorganisms were observed as circular shapes, approximately 0.2 to 0.6 µm wide and up to 1.6 µm in length. The limiting boundary was formed by two clearly defined structures: the rickettsial cell wall, which surrounds the whole microorganism, and the underlying plasma membrane, which limits the cytoplasm. Sections that were taken perpendicular to the cell wall and plasmalemma revealed the total thickness of the rickettsial interspace to be approximately 200 to 250 Å. The cell wall was approximately 90 Å thick, and the plasma membrane thickness was approximately 75 Å (Fig. 3B).

DISCUSSION

The present work investigated the cause of juveniles yellow clam (*Mesodesma mactroides*) mortality event that occurred in October of 2011 at an intertidal bed in Cassino Beach, State of Rio Grande do Sul, southern Brazil. Herein, no adults of yellow clam were recorded in the sampling area. According to Sindermann (1990), infectious diseases are common suspects in shellfish mortalities. In this study, we provide the first report of prokaryotes of the genus *Rickettsia* in the gill and intestinal epithelial cells of *M. mactroides*.

The order of Rickettsiales pertains to the Alphaproteobacteria class and is composed of microorganisms with an obligate intracellular and parasitic lifestyle (Dumler & Walker, 2005). This group of prokaryotes is best known for its medically important genus *Rickettsia*, whose species can cause severe and occasionally fatal diseases in man (Parola & Raoult, 2001). The ultrastructural characteristics of rickettsiae were clearly recorded in the present study. In thin sections, the microorganisms are seen as circular or rod-shaped profiles. The limiting boundary is formed by two clearly defined structures: the rickettsial cell wall, which surrounds the whole microorganism, and the underlying plasma membrane, which limits the cytoplasm. The contours of the rickettsiae are generally smooth, with few irregularities. Some specimens show wrinkled or bulbous outpocketings of the cell wall. The plasma-

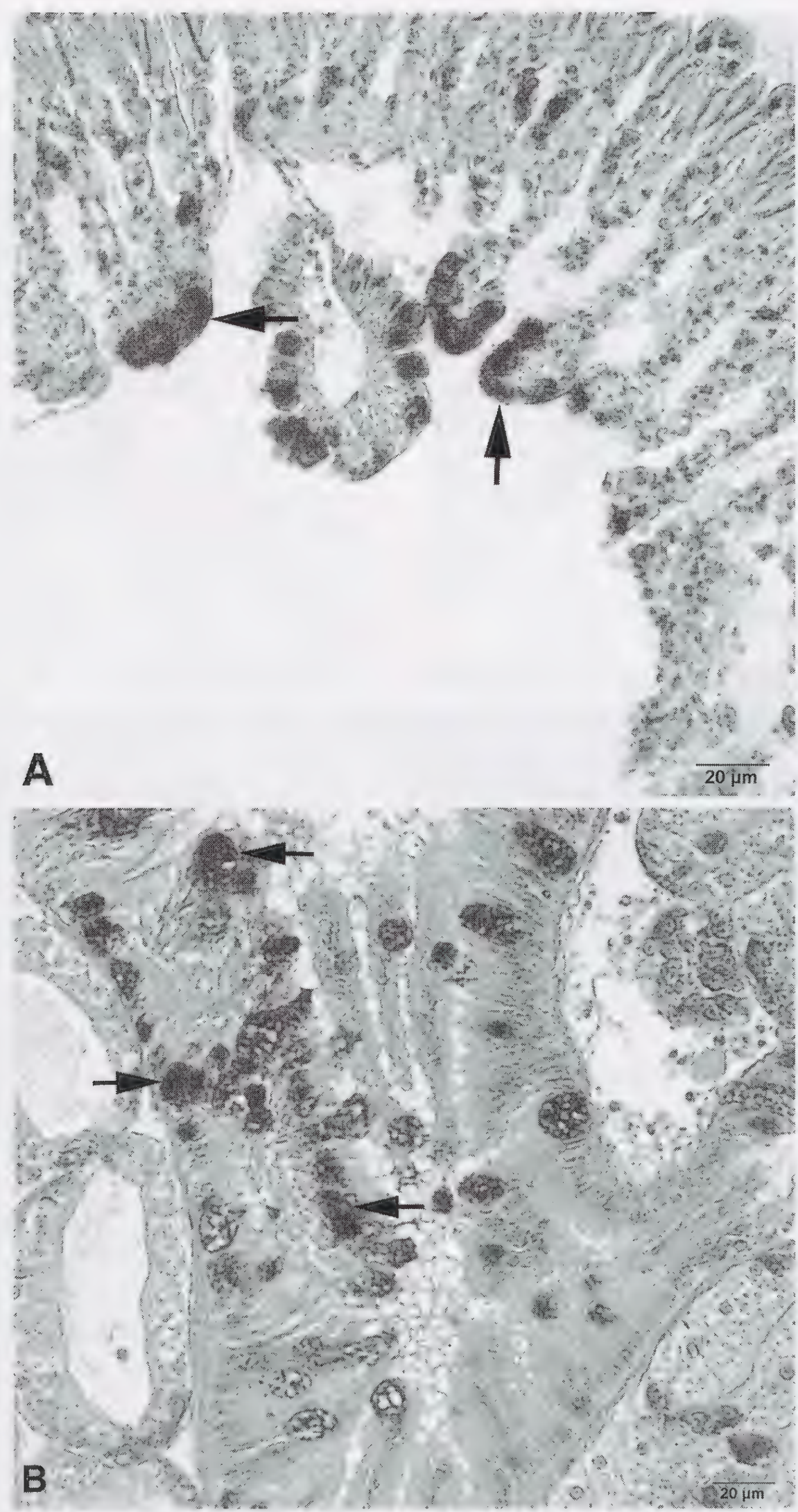


FIG. 2. *Mesodesma mactroides*. FIG. 2A: Gill of showing basophilic inclusions (arrows). Hematoxilin-Eosin staining; FIG. 2B: Intestinal epithelium with basophilic inclusions. Hematoxilin-Eosin staining.

lemma has occasional vesicular inpocketings, these invaginations resemble the pinocytotic vesicles or vacuoles seen in animal cells. Their contents are of low electron density and are continuous with the structure less clear space between the plasma membrane and the cell wall. At the site of these invaginations, the

cell wall is uninterrupted and does not parallel the infolded plasmalemma (Robertson, 1959; Cheville, 2009)
The first record of rickettsiae in bivalves was in 1977 in the soft shell clam, *Mya arenaria* from Chesapeake Bay, U.S.A. (Harshbarger et al., 1977). Since then, rickettsiae have been

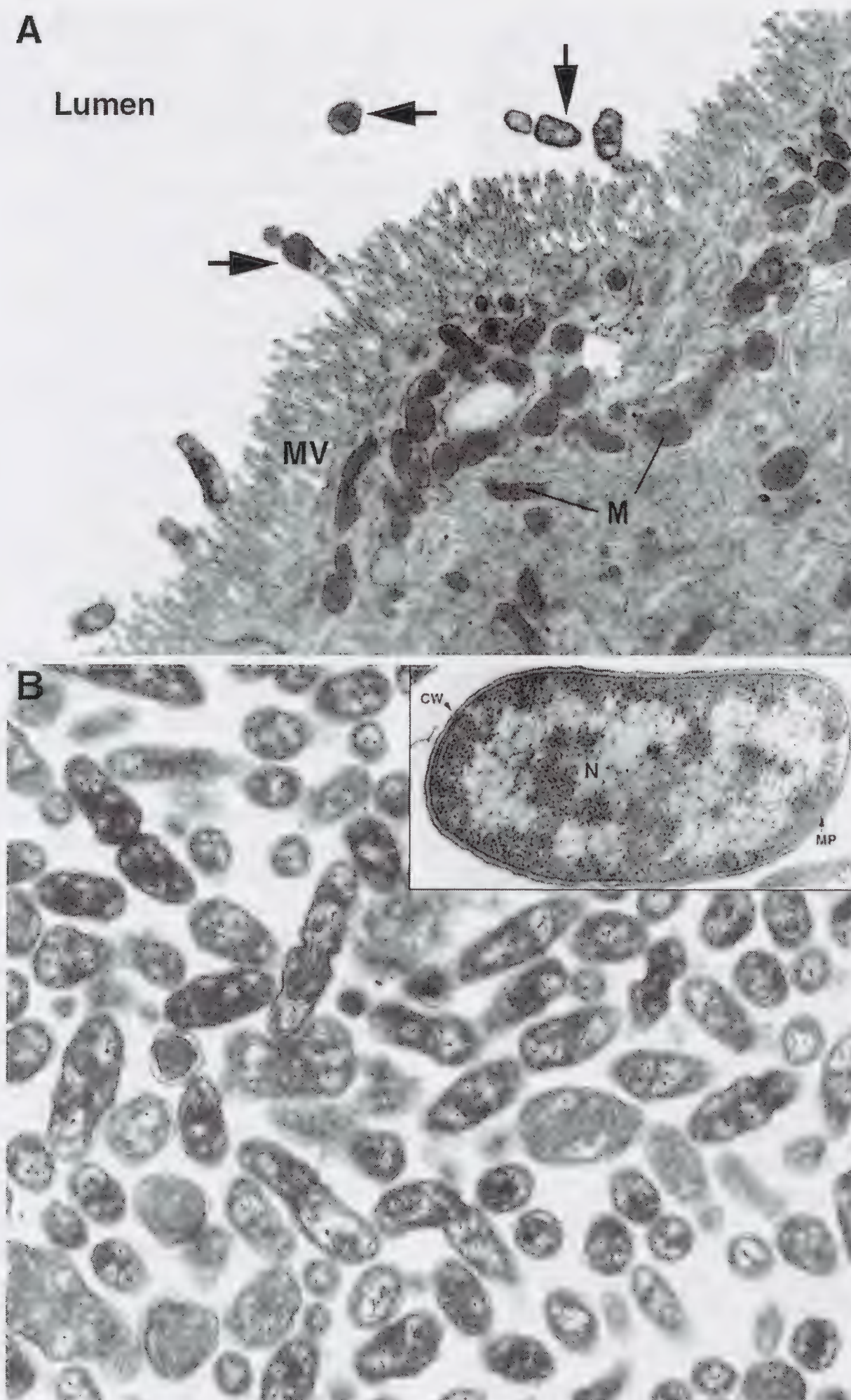


FIG. 3. Electron micrographs of *Mesodesma mactroides* and *Rickettsia*. FIG. 3A: Intestinal epithelium of *Mesodesma mactroides*. The lumen contains several rickettsiae (arrows); some are in close association with the microvilli (MV). The dense mitochondria in the epithelial cells have a different internal structure than the extracellular microorganisms (M). X 9,500; FIG. 3B: Central portion of a colony of *Rickettsia*. Circular profiles represent transverse sections, and elongate forms are sectioned longitudinally. Note the constriction furrows across the centres of several rickettsiae. With the exception of several pleomorphic forms, the colony is remarkably homogeneous. X 24,000. Insert: *Rickettsia* cells from the intestine of an infected *Mesodesma mactroides*. Both cell wall (CW) and plasma membrane (MP) are trilaminar when viewed on edge. The nuclear component (N) is represented as areas with fine filaments. X 125,000.

reported in several bivalve species, and they predominantly inhabit in the epithelia of the host's intestines and gills (Buchanan, 1978; Elston & Peacock, 1984; Mialhe et al., 1987; Fries & Grant, 1991). Members of the order Rickettsiales have previously been associated with mortalities of natural populations of different invertebrate groups (Sparks, 1985).

Some studies claim that *Rickettsia* infections cause little harm to bivalves as too few cells become infected (Harshbarger et al., 1977; Bower et al., 1994; Cremonte et al., 2005; Boehs et al., 2010). Indeed, when *Rickettsia* infection occurs in high intensity, it has been associated with significant mortalities in bivalve populations (Buchanan, 1978; Lauckner, 1983; Elston, 1986; Le Gall et al., 1988; Norton et al., 1993; Villalba et al., 1999). Bivalve larvae and juveniles are more vulnerable to prokaryote infections than adults as stated by Kinne (1983) and Elston (1999). In other invertebrates and vertebrates, rickettsiae that invade the intestinal epithelial cells and produce an alteration in nutrient absorption (Walker et al., 2003). It may also happen in *Mesodesma mactroides*.

The first description of a Rickettsiales-like organism (RLO) in the Brazilian aquatic fauna was recorded by Azevedo et al. (2005). They found RLOs infecting the gill epithelium of the mangrove oyster, *Crassostrea rhizophorae*, in the State of Piauí in northeastern Brazil. They suggested that RLOs could be associated with mortality of the parasitised oyster due to the disappearance of the apical cilia with concomitant lysis of the parasitised epithelial cells of the gill. Da Silva et al. (2011), and Sabry et al. (2011) found RLOs in very low prevalence in epithelial cells of the digestive glands of oysters (*Crassostrea gigas* and *C. rhizophorae*) from Santa Catarina State in southern Brazil, and those cells did not sustain serious damage.

This is the first report of *Rickettsia* infection among *Mesodesma mactroides* and the first histopathological survey of diseases of marine bivalves from Rio Grande do Sul State. We observed that 100% of the bivalve specimens analysed were infected with *Rickettsia*. Furthermore, the specimens were moribund at the time of collection, and upon returning to the sampling site for observations, it was noted that all juveniles were dead. Therefore, our data suggests that *Rickettsia* contributed to the mortality event of *M. mactroides* reported in Brazil in 2011.

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